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Ustilago Crameri Koern.

ON

Setaria italica Beauv.

BY

S. SUNDARARAMAN, M.A.,
Acting Government Mycologist, Madras.

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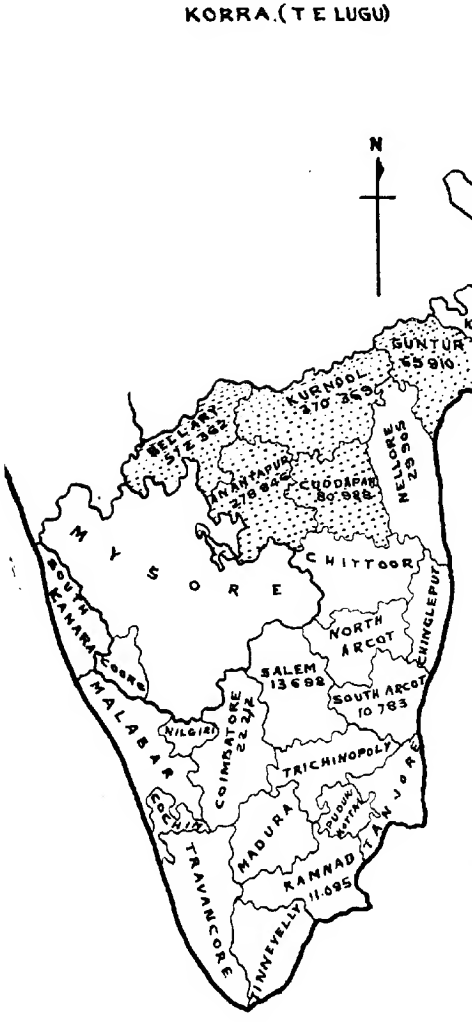
MADRAS PRESIDENCY.

AREA UNDER.
ITALIAN MILLET.(SETARIA ITALICA).
TENAI.(TAMIL)
KORRA.(TELUGU)



DISTRICTS	AC
GANJAM	2
VIZAGAPATAM	1
GODAVARI	
KISTNA	
GUNTUR	6
KURNOOL	37
BELLARY	57
ANANTAPUR	27
CUDDAPAH	8
NELLORE	2
CHINGLEPUT	
SOUTH ARCOT	1
CHITTOOR	
NORTH ARCOT	
SALEM	1
COIMBATORE	2
TRICHINOPOLY	
TANJORE	
MADURA	
RAMNAD	1
TINNEVELLY	
MALABAR	
SOUTH KANARA	
THE NILGIRIS	
TOTAL:	1.52

Ustilago Crameri Koern. on *Setaria italica* Beauv.

[*Received for publication on 18th March, 1920.*]

Setaria italica is grown in all the districts of the Madras Presidency except Malabar and South Kanara. It is called 'Tenai' in Tamil and 'Korra' in Telugu. The total area of this crop in 1918 was 1,529,187 acres. The northern central districts of Cuddappah, Kurnool, Bellary, Anantapur and Guntur among themselves accounted for an area of 1,368,575 acres which is nearly 90 per cent of the total. Most other districts raise this crop to a greater or less extent wherever rain conditions permit. The importance of the crop as a food is well known. The map shows the extent of its cultivation in the several districts. It is grown as a pure crop in limited areas, but is generally grown mixed, the chief crop associated with it being cotton. In fact, this has given a name to the mixture which is known as "Korrapatthi" in the Ceded Districts.

Setaria italica is subject to the attack of some fungus diseases which cause much damage, the most important being *Sclerospora graminicola* Schroet (green-ear disease), *Uromyces Setaria italicæ* Dict. (rust) and *Ustilago Crameri* Koern. (smut). In the flowering stage the green-ear disease fungus causes proliferation of all parts of the flower so that the earhead becomes a mass of leafy shoots. The rust fungus produces brown spots on the leaves. The one that causes by far the greatest loss, however, is smut which converts the valuable food stored as starch in the seeds into a mass of black powder.

Symptoms. Outwardly the grains are the only parts that appear infected. The smutted grains are only very slightly larger than healthy ones. In the early stages when the healthy grains are still green, the smutted ones have become grey, while at a later stage the former become yellow, while the latter are brown. When the membrane of the smutted grain bursts (Pl. I, figs. 1 and 2), the black spores are liberated and give the grain and the glumes a dark dusky appearance, readily seen and much dreaded in certain tracts, where the cultivators aptly call it *katika korra* or sooty millet, though when pressed between the fingers, the

spore mass is not jet black but slightly brown. The spores are formed only in the spikelets. Sometimes all the shoots of the plant are affected and almost all the grains in the ear may be attacked. In some cases the upper spikelets may be free. In a partially attacked earhead the tip may be sound but the base is always affected. In no case has the upper portion of the earhead been found diseased, while the lower was sound. This gives an indication that infection proceeds from the bottom upwards and that when the inflorescence began to elongate the fungus did not keep pace with the development of the inflorescence and did not reach the uppermost part of the panicle.

Origin. In Siruguppa Taluk of Bellary District the disease was very severe in 1917, and a report by the Agricultural Demonstrator there led to the investigation of this subject. The grains of ten smutted earheads sent to the Laboratory were counted with a view to find out the proportion of healthy to smutted grains. There were 2,261 healthy grains and 9,005 smutted grains. The proportion of healthy to smutted grains was 1 to 4. Details are given below :—

No.	Healthy grains	Smutted grains
1	115	314
2	123	580
3	237	678
4	488	1,232
5	34	789
6	89	892
7	587	1,352
8	25	684
9	58	589
10	525	1,895
TOTAL	2,261	9,005

Loss. The area under Italian millet in Siruguppa Taluk in 1918 was 23,800 acres. The estimated loss because of smut was more than a quarter of the crop : but if it be taken at a quarter of the crop and

the average yield at 1,000 lb. per acre, then the total loss for the Taluk would be 250 lb. per acre, which at the price current at the time amounts to a money value of 11,90,000 rupees. The area under this crop in Siruguppa Taluk is only one-sixtieth of the total area of the Presidency, and though the smut was not so severe in most other tracts still it was present to some extent in all and this gives an idea of the great loss caused by this smut in one year. It thus became important that a method of treatment suitable to the means and capabilities of the ryot should be devised and demonstrated in those tracts where smut was severe and recurring.

Spores. In bulk the spore masses are black but when in a thin layer they look dull brown. They are aggregated into spore-balls (Pl. I, fig. 3) which readily disintegrate under a cover slip into the component spores. In shape they are globular to ovoid, and some show irregular indentations (Pl. I, fig. 3). They are smooth and their contents appear more or less granular. They measure 7·7—11·1×5·3 - 8·2μ.

When placed in water, the spores begin to germinate usually in 6 to 9 hours. A germ tube is produced and the protoplasm contained in the spore passes into the tube (Pl. I, fig. 4). The rupture of the wall of the spore is so pronounced that the projecting portions of the spore-wall are seen along each side of the base of the germ-tube. It becomes divided by three or four septa (Pl. I, fig. 4). From each of the cells small buds arise near the septum and form sporidia. These, when detached, may either put forth a germ tube or two adjacent sporidia may become connected by a transverse branch. There are instances where the promycelium may not develop sporidia but becomes an ordinary hypha and penetrates the tissue of the host plants by its pointed extremity. Again a kind of fusion takes place by means of a tube from one segment of the same promycelium. Thus the upper segment becomes connected with the lower segment of the same promycelium by means of a curved tube which Brefeld[1] calls a "buckle-joint" or "knee-joint" (Pl. I, fig. 4, a, b, c). This is common when spores are germinated in water. But when germinated in sugar solution, these fusions do not occur, but the sporidia multiply in the manner of budding of "Yeast," thus giving rise to copious sporidial formation. This goes on until all the nutritive medium is exhausted. The course of infection is as follows :—

Penetration. The germ tube penetrates the wall of an epidermal cell and grows through the cells beneath the surface layer (Fig. 5). In

1 Brefeld. *Untersuchungen aus dem Gesamtgebiete, etc., der Mykologie.* Vol. V, 1883, pages 101 to 102.

Germination of the spores of this smut has been dealt with in detail.

the early stage of the seedling the hyphæ in sections of the nodes, are very numerous and very clearly seen. A month after the infection, hyphæ are only seen in the nodes (and near the apex of the shoot) (Pl. II). The fungus is found in masses. Perhaps this is due to the fungus not being able to keep pace with the rapid growth of the plant and the fungus gets torn up and isolated into fragments. Infection takes place when the seed begins to germinate and penetration has been seen only through the epidermal cells of the hypocotyl. The young mycelium grows inside along with the seedling, showing no external signs of its presence until the earheads are produced, when it forms its spores in the ovary.

Prevention—local. The seriousness of this disease and the necessity for its prevention are not unknown to the ryot of the Ceded Districts, only the steps taken are primitive. He thinks that a mixture of cattle-urine, garlic, asafœtida, sweet-flag, is effective, but this has been on various trials found to be ineffective. Various preventive measures[1] are used against this and similar smuts on cereals in other parts of the world as washing the grains in water, steeping the grain in copper sulphate solution or in formalin or in hot water or exposing it to hot air. Copper sulphate is known to the ryots and is available in the local bazaar. It is the cheapest, safest and most acceptable method so far as he is concerned and hence it is recommended. Formalin, though as effective as copper sulphate, is difficult to handle except under skilled supervision. The hot water, and the hot air methods, however, are not suitable to the ordinary cultivator, as he has no knowledge of the meaning or use of a thermometer. The limits of safety were considered too narrow for the satisfactory adoption of this method.

Other references.

1. G. P. Clinton in "North American Ustilagineæ" has described this smut on the Italian millet as *Ustilago Crameri* Koern. His description is "Sori in the spikelets, infecting all of the spike, ovate, about 2-4 mm. in length, chiefly destroying inner and basal parts; spores reddish-brown, chiefly ovoid to subspherical though occasionally more elongated and irregular with usually pitted contents, chiefly 8-11μ in length.

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[1] Hecke, L. Vorversuche zur Bekampfung des Brandes der Kolbenhirse. *Zeits. Landw. Ver. Oesterr.*, 1902, 933, 1902.

(Gives results of treatment with hot water, formalin, corrosive sublimate, and copper sulphate on germination of *Ustilago Crameri* and *U. Panici-miliacei*.)

Stuart, W. Formalin as a Preventive of Millet Smut. *Ann. Rep. Ind. Agr. Exp. St.*, 13, 25, 1901, shows from experiment that seed soaked 1-4 hrs. in formalin, rate of 1 lb. to 45 gals. of water, was effective in preventing this smut.

"This fungus sometimes causes damage to the cultivated millets. It has been reported only during recent years from this continent. The ordinary hot water method of seed treatment is a preventive."

2. Brefeld. *Untersuchungen aus dem Gesamtgebiete, etc., der Mykologie*, Vol. V, 1883, pages 101 to 102.

Germination of the spores of this smut has been dealt with in detail.

3. Hecke, L. Vorversuche zur Bekampfung des Brandes der Kolbenhirse. *Zeits. Landw. Ver. Oesterr.*, 1902. 933, 1902.

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5. Butler, E. J. "Fungi and disease in plants," pages 234-235.

As is the case with *Cintractia Sorghi Vulgaris* on *Andropogon Sorghum* (Short smut on cholum *Tamil* and jonna *Telugu*), another smut known to the cultivator and whose method of prevention is gradually being adopted by them, the smut on Italian millet, also yields readily to preventive treatment. The method of infection in both cases is the same. As in *Sorghum*, the smut on Italian millet adheres to the surface of the seed, germinates along with the seeds and enters into the young seedling only during the seedling stage.

Preventive experiments. In the investigation of this preventive method, the points to be settled were :—

1. the concentration of copper sulphate solution which would undoubtedly prevent the germination of the spores,
2. whether that concentration of the solution would at all affect the germination of the seed, and
3. whether the results obtained by 1 and 2 were confirmed by field trials.

1. *The effect of copper sulphate solution on the spores.* A few smutted grains were broken up and a small quantity of spores collected and made into ten lots for treatment. Each lot was put in a test tube. A few spores from each lot were placed on slides in distilled water to germinate. In 24 hours almost every spore had germinated in all the eleven lots.

The tubes with the spores were thoroughly shaken several times during the period in which they were in solution and then left to settle. The fluid in each tube was removed with a pipette and distilled water

put in. The tubes were again well shaken. When the spores again settled down the water was taken off and the tube refilled with water. This rinsing was repeated a number of times until the last rinsing water contained no trace of copper sulphate. As a further test, the last rinsing water from each tube was poured into a tube containing spores and left for the corresponding period of time. In no case did this steeping in this rinsing water affect the germination of the spores. The details of the experiment are given in the tabular form.

Test tube No.	Concentration of copper sulphate solution used	Time soaked	Period taken for testing germination	Results
1	..	12 hours	12 hours	In distilled water 50 per cent germination in 6 hours, 95 per cent in 12 hours, and 100 per cent in 24 hours.
2	0·1 per cent	15 minutes	120 hours	About 50 per cent germinated in 24 hours. The germ tube of spores that germinated lengthened and even this stopped after 96 hours.
3	0·1 „	30 „	Do.	No germination.
4	0·5 „	15 „	Do.	Do.
5	0·5 „	30 „	Do.	Do.
6	1·0 „	15 „	Do.	Do.
7	1·0 „	30 „	Do.	Do.
8	2·0 „	15 „	Do.	Do.
9	2·0 „	30 „	Do.	Do.
10	5·0 „	15 „	Do.	Do.

Note.—6 slides with drops of distilled water were used for germinating the spores in each case.

From these experiments it is seen :

(1) that the spores germinated in distilled water to the extent of 50 per cent in 6 hours and freely in twelve hours ;

(2) that 0·1 per cent solution of copper sulphate for 15 minutes retarded germination and hindered growth: the promycelium was short and did not produce sporidia during the period of observation ;

(3) that 0·1 per cent for 30 minutes stopped germination entirely as did all higher concentrations for periods of 15 and 30 minutes.

Under the conditions of the experiment, in working with small quantities of spores it is likely that the spore masses will be broken into their component parts to a greater extent than they will be in a field experiment where the seed is steeped in a copper sulphate solution. It may be anticipated that the spores may not be so well separated from each other and that the copper sulphate solution may thus not get at every individual spore, with the result that the same effect may not be produced in the field with any one concentration as is shown in the table of results.

2. *The effect of copper sulphate solution on the germination of the seeds.* In each experiment 100 seeds were placed in moist sterile sand. The sand was sterilized by being kept in hot-air sterilizer at 170 degrees centigrade for half an hour. In 48 hours all the 100 seeds germinated, showing thereby that the seeds were in a very good condition. With the seeds thus tested the following 15 series of experiments were tried with the results tabulated below :—

Serial No.	Strength of copper sulphate solution	Time steeped	Percentage of germination	Number of hours taken for germination
1	Untreated seed	...	100	48
2	0·5 per cent	15 minutes	99	96
3	0·5 „	30 „	100	120
4	1 „	15 „	98	Do.
5	1 „	30 „	99	Do.
6	2 „	15 „	99	Do.
7	2 „	30 „	99	Do.
8	2 „	10 hours	99	192
9	5 „	15 minutes	95	Do.
10	5 „	30 „	92	Do.
11	10 „	15 „	79	Do.
12	10 „	30 „	84	216
13	15 „	15 „	63	Do.
14	15 „	30 „	69	Do.

The experiment shows that the lower concentrations have no effect on germination, that the lowest concentration that has a definite effect is 5 per cent and that after steeping for 30 minutes, and that, above that

concentration, the germination is lowered in proportion as the concentration is greater. It is thus obvious that 5 per cent concentration of copper sulphate solution is the limit within which we can safely steep seeds. However, smut spores fail to germinate with even 0·5 per cent copper sulphate solution for 15 or 30 minutes. As a working formula, therefore, the strength of solution which is required to kill the spores and at the same time not affect the germination of the seed may be taken at somewhere about 2 per cent and the ordinary length of time may be half an hour. This allows a large limit for possible errors both in making the solution in the rough way in which a cultivator does it and in the time of steeping and still ensures success to the treatment.

Test as to whether the results obtained by (1) and (2) are confirmed by making a trial on a field scale. Seed was obtained from the stores at the Central Farm, Coimbatore. In order to get clean seed for this experiment it was steeped in 2·5 per cent formalin (*i.e.*, Schering's formalin diluted) for 15 minutes. That this treatment completely destroys the spores but does not hinder the germination of the seed was proved by repeated experiments. 32 plots were laid out in the compound of the Pot-Culture house of the Government Mycologist and were sown with seeds treated in the manner detailed below :—

1. Seed as obtained from the Central Farm stores without any treatment k. 1.
2. Seed soaked in 2·5 per cent formalin solution for 15 minutes k. 2.
3. Part of k. 2 was mixed with spores of *tenui* smut and this is k. 3.
4. Part of k. 3 was treated with copper sulphate solution as detailed below :—

Series	Strength of copper sulphate solution	Time of soaking
4	0·5 per cent	15 minutes (k. 4)
5	0·5 ,,	30 ,, (k. 5)
6	1 ,,	15 ,, (k. 6)
7	1 ,,	30 ,, (k. 7)
8	2 ,,	15 ,, (k. 8)
9	2 ,,	30 ,, (k. 9)
10	2 ,,	10 hours (k. 10)
11	5 ,,	15 minutes (k. 11)
12	5 ,,	30 ,, (k. 12)
13	10 ,,	15 ,, (k. 13)
14	10 ,,	30 ,, (k. 14)
15	15 ,,	15 ,, (k. 15)
16	15 ,,	30 ,, (k. 16)

Duplicate plots were laid out in each case, the seeds were sown on 20th November, 1918, and harvested on 13th February, 1919. The

plots that were sown with seed infected with smut, *i.e.*, (k. 3), showed 208 smutted (earheads) plants out of 350 that grew. All the other plots were entirely free from smut.

Thirty-two plots of one-cent each were laid out in field No. $2c_2$ in the Government Agricultural Station, Hagari, with the kind co-operation of Mr. Hilson, Deputy Director of Agriculture, Circles II and III. The seeds were sown on 23rd September, 1918. The plots were harvested in January, 1919. Only the artificially infected plot produced smutted earheads. The germination in all the plots was for some reason very uneven and the experiment is taken only qualitatively.

The following experiments were made to determine the place in the *tenai* plant through which the smut gains entrance. *Tenai* seed obtained from the Agricultural Demonstrator, Bellary, was disinfected with 2·5 per cent formalin for 15 minutes, dried, smeared with spores of the smut, and sown in pots with sterilized soil on the 17th December, 1917. Another lot of seeds was also sown at the same time, but these were disinfected with formalin but not smeared with smut spores. The pots used were new and were immersed in a 0·1 per cent solution of corrosive sublimate and dried. The soil that was put in the pot was kept in small lots in a hot-air sterilizer at a temperature of 170 degrees centigrade for half an hour. These pots were watered with sterilized water till the seeds came up to a seedling stage. When seeds germinated, some of them were carefully removed from the pots and examined under the microscope. But spores were seen to have germinated and to have produced lots of sporidia in two days. The germ tubes from the germinating spores were seen on the surface of the seed-coat and on the epidermal layer of the plumule and of the hypocotyl. The course of infection is as follows. The germ-tube penetrated the wall of the epidermal cell of the hypocotyl (Pl. I, fig. 5) and grew through the cells beneath the surface layer. In the early stages of the seedling, hyphæ are seen to be very numerous in sections of internodes and nodes. A month after infection, however, the hyphæ were only seen in the nodes, in the form of masses. Infection takes place when the seeds begin to germinate and the young mycelium grows along with the seedling showing no external signs of its presence until the flowers are produced, when it again forms its spores in the ovary. The seedlings grew up under sterile conditions in the pots for about a month. When they had grown sufficiently high they were transplanted into big pots. The soil used to fill up the pots was obtained from the Central Farm, Coimbatore. Sections from the nodes and internodes of some seedlings showed thick nodulose hyphæ, Pl. II. Out of 100 plants in the pots 95 showed smutted earheads. In the 100 plants grown out of seeds

that were not smeared with smut, no smutted earhead was seen. In a field in the Central Farm Coimbatore, sown on the 17th November, 1917, and harvested on the 5th February, 1918, out of 300 plants that grew from seeds smeared with smut spores, 165 plants showed smutted earheads. In the 300 plants that grew from seeds not smeared with smut spores, not a single plant was smutted.

To determine if the spores of the smut infect flowers of Italian millet and produce smut on the ears, the following experiment was laid out.

Tenai seed was disinfected with 2·5 per cent formalin for 15 minutes, dried, and sown in pots. The pots were new and dipped in 1 to 1,000 parts of corrosive sublimate solution and the soil sterilized by being kept in a hot-air sterilizer at 170 degrees centigrade for half an hour. The experiment was divided into two series with 20 plants in each series. When the plants grew up and were in shot-blade, the tops of these 40 plants were covered over with paper bags. As soon as the earheads came out of sheathing leaf, the stigmas of the flowers were dusted with smut spores in the following way. Dry spores of the smut were placed in a paper bag and shaken on to the flowers by inverting the bag over the earhead and shaking thoroughly. Sometimes the spores were placed with a camel hair brush. Examination of the dusted earheads with a hand-lens showed spores on the stigmas of flowers. The other set of 20 plants that were bagged, were not interfered with in any way, *i.e.*, they were not dusted with spores. After the earheads matured, the paper bags were removed and none of the earheads in either of the two sets were found to be smutted. This was repeated three times. It is thus seen that the spores of the smut are not capable of infecting the flowers of the plant. It may sometimes be suggested that the spores may lie dormant in the soil for one season and affect the later crop. This is against the life-history of the smut as the spore germinates quickly in water in a short time and the field is likely to have received a shower or two in the fallow period.

From the foregoing experiments the limits for copper sulphate solution both with regard to the fungus and the seed have been determined to be 0·5 per cent and 5 per cent, and 2 per cent has been shown as a good working concentration. The formula is easily translated into the measures understood by the ordinary cultivator. A kerosene bottle ordinarily available in the bazaar can be taken as a measure for water, and a rupee as the weight for the copper sulphate. To secure 2 per cent strength of solution he has to dissolve a rupee weight of copper sulphate in a bottle of water. The cultivator has thus to remember only two things, *i.e.*, one rupee weight of copper sulphate and a kerosene oil bottle of water. Steeping in 2 per cent solution for 10 minutes has been re-

commended in Bombay. But the bulk of the ryot population have yet to become familiar with the means of gauging accurately sub-divisions of an hour. The ryot has a rough and ready method of determining the time. Generally he keeps no clock or time-piece. He is guided mostly by the shadow cast by an object and he is always very nearly correct. He has inherited this method from time immemorial and it has not failed him: and a method he would understand better is to be perferred. Half an hour is easily understandable, a better unit which leaves a sufficient margin of safety.

The Deputy Director of Agriculture of Circles II and III conducted field trials with seed treated with 2 per cent copper sulphate solution. This was done consecutively for three years. A definite set of instructions was prepared for guidance and Agricultural Demonstrators visited villages several times before and after sowing. They showed the people how to prepare the solution and how to treat the seed in solution. In some plots treatment of seed and sowing were done under their personal supervision, while, in the majority of cases, the ryots did this part of the work closely following their instructions. During the several inspections of the treated plots by the Agricultural Demonstrators it was found that the treated plots presented no appearance of smut. This was the case not in one or two plots, but in hundreds of plots. One may realize the efficacy of this treatment, when one realizes that in 1917 ryots, usually extremely conservative, had grown 9,312 acres in Bellary District alone with treated seed. At the same time, it is necessary that only good seeds should be used as old grain may be injured by the treatment. The seeds should be dried quickly or sprouting may result. Sowing should be done soon after treatment.

SUMMARY.

(1) Smut causes great loss in *Setaria italica* (Italian millet) in certain districts of the Madras Presidency.

(2) The spores germinate freely in distilled water to as high a percentage as 95 in twelve hours; but fail to germinate when placed in solutions of copper sulphate of 0·5 per cent and higher concentration for 15 to 30 minutes.

(3) That steeping seed of *Setaria italica* in solutions of copper sulphate of 5 per cent concentration for 30 minutes and higher concentration for 15 to 30 minutes reduces germination.

(4) That the smut enters the plant in the young seedling stage.

(5) That the spores do not infect the flowers.

(6) That to secure safety from infection, seed should be steeped in a 2 per cent solution of copper sulphate for 30 minutes.

EXPLANATION OF PLATE I.

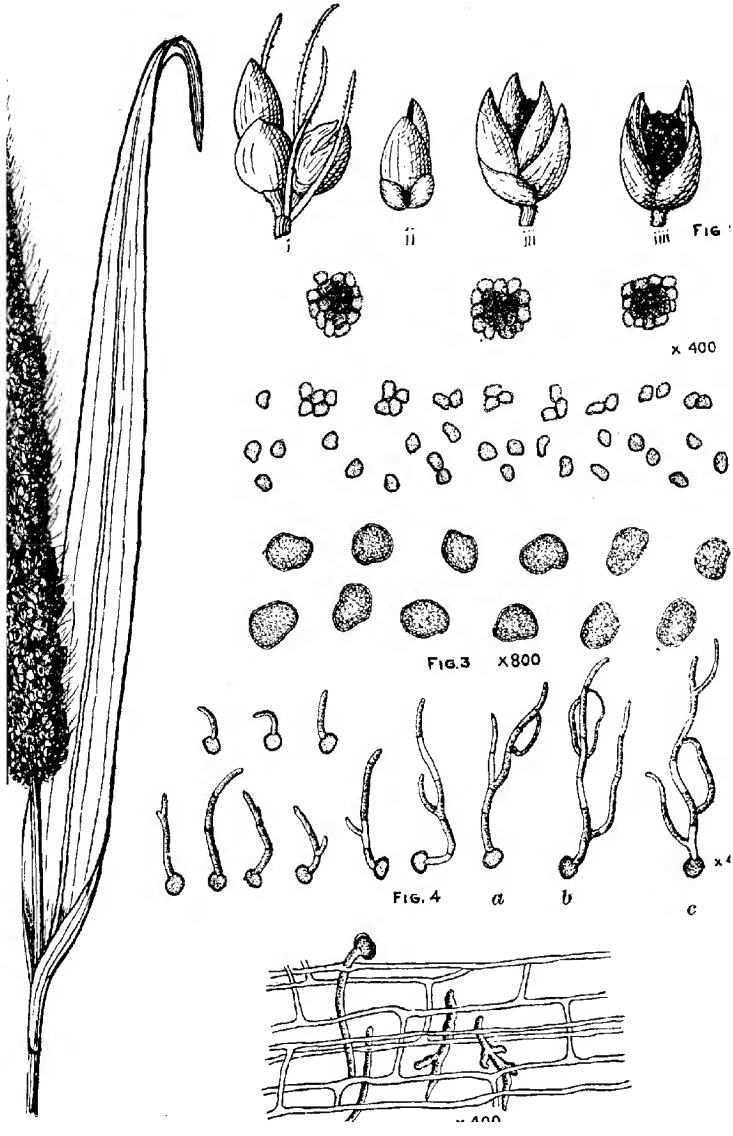
Fig. 1. Earhead of *Setaria italica*, Italian millet, with smutted grains.

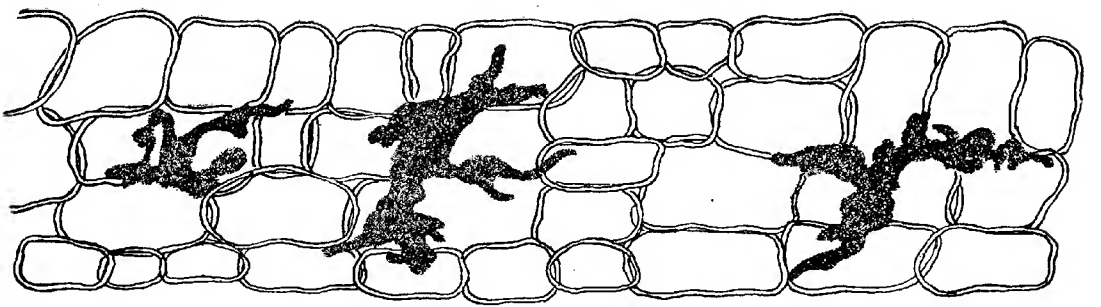
„ 2. Smutted grains enlarged.

„ 3 Spores of *Ustilago Crameri* Koern. × 400 and × 800.

„ 4. (*a*) Germinating spores in different stages.
(*b*) Promycelia showing the "buckle-joint" × 400.

„ 5 Germ-tube entering the epidermal cells of the young hypocotyl of the millet seedling × 400.


i
ii
iii
iiii
FIG
x 400
FIG.3 x800
FIG. 4
a
b
c



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